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The effect of cyanobacterial blooms in the Siemianówka Dam Reservoir on the phytoplankton structure in the Narew River

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Abstract

The effect of cyanobacterial blooms on the phytoplankton structure in the lowland Narew River (north-eastern Poland) was examined. The studies were carried out at stations situated at different distances from the eutrophic Siemianówka Dam Reservoir. In 2008, the investigated lowland reservoir and the outflowing river were characterized by the dominance of toxic cyanobacteria (from July to October). At a station situated 130 km below the dam, species composition in the river was very similar to that in the shallow reservoir. *Planktothrix agardhii* was the main and permanent dominant, both in limnoplankton and potamoplankton. The current study indicates that the eutrophic Siemianówka Dam Reservoir is the main and rich source of phytoplankton for the outflowing Narew River. Cyanobacteria were dominant in the river phytoplankton at all sampling stations, but their share in phytoplankton biomass gradually decreased with the distance from the dam. Chemical analysis revealed the presence of microcystins (MCs) in water samples collected from June to October. The toxins, mainly demethylated MC analogues, were detected at a long distance down the river,

even 100 km from the dam. Maximum concentration of MCs (14.3 µg l⁻¹) was measured on 13 October, 9.1 km below the dam.

INTRODUCTION

In most large European rivers, the phytoplankton community is dominated by diatoms throughout the year, whereas share of other groups increases significantly only during shorter periods (Skidmore et al. 1998, Everbecq et al. 2001, Karrasch et al. 2001, Sabater et al. 2008). Relatively high biomass of cyanobacteria in potamoplankton has been observed in eutrophic rivers during periods with low discharge and flow velocity. Stronger development of riverine cyanobacteria has been observed mainly in summer and/or autumn (Bahnwart et al. 1999, Köhler and Hong 2000, Szelaż-Wasilewska et al. 2009), whereas in Elbe also in winter (Karrasch et al. 2001). Although cyanobacterial blooms typically occur in lakes and other reservoirs, some incidents of mass cyanobacterial developments were recorded in rivers, as well. They took place during periods of low flow and thermal stratification. In Australia, the blooms of *Anabaena* occurred in the River Murray-Darling, and Murrumbidgee (Mitrovic et al. 2003). Some of the blooms contained saxitoxin produced by *Anabaena circinalis*.

Construction of a dam modifies the biogeochemical cycles by changing the nutrient balance and residence time of river waters, altering oxygen and thermal conditions (Malatre and Gosse 1995, Köhler and Hong 2000, Friedl and Wüest 2002, Jekaterynczuk-Rudczyk and Górniak 2006). Longer retention of riverine water in the reservoir causes a significant increase of phytoplankton biomass (Grabowska 2005, 2006b). Numerous incidents of human and animal poisoning in consequence of cyanobacterial blooms in dams were described by Kuiper-Goodman et al. (1999). The consequences of altered processes and hydrological

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parameters can be observed over long distances from the dam (Malatre and Gosse 1995).

In flushed systems, the phytoplankton structure is determined by physical factors like retention time of water (Köhler and Hong 2000, Everbecq et al. 2001, Sabater et al. 2008), light condition (Everbecq et al. 2001, Reynolds 2003), tributaries (Skidmore et al. 1998, Karrasch et al. 2001), flow velocity (Skidmore et al. 1998, Bahnwart et al. 1999, Salmaso et al. 2008) and changing nutrient supply (Köhler and Hong 2000, Sabater et al. 2008).

There is an increasing number of reports on phytoplankton development in rivers. However, cyanobacteria and their toxins have been studied more frequently in lentic ecosystems (Fastner et al. 1999; Kuiper-Goodman et al. 1999; Pawlik-Skowrońska et al. 2004, 2008; Mazur-Marzec et al. 2008; Grabowska and Pawlik-Skowrońska 2008; Kabziński et al. 2008) than in lotic ones (Mitrovic et al. 1999, Szeląg-Wasilewska et al. 2009).

The aim of this work was to study the structure of phytoplankton in the Narew River at different distances from the Siemianówka Dam Reservoir. Additionally, microcystins – cyanobacterial peptide hepatotoxins – were analyzed both in the eutrophicated dam reservoir and the river below the dam.

MATERIALS AND METHODS

Study area

In the north-eastern part of Poland, the Narew River (NR) is the largest river in the catchment of the Vistula River. This lowland river drains the periglacial sandy-loam catchment with different percentage of forest and wetlands. The upper part of the Narew River is characterized by a peat-covered catchment. Multiannual discharge of the Upper Narew River varies from 5 to 21 m³ s⁻¹ (Zieliński et al. 2003). The Siemianówka Dam Reservoir (SDR) (52°55'N, 23°50'E) is a shallow polymictic reservoir constructed in the Upper sector of the Narew River in 1990 (Fig. 1). In the phytoplankton of the dam reservoir, strong permanent dominance of potentially toxic cyanobacteria has been observed in summer and autumn, starting from the early existence of the reservoir (Grabowska 2005, 2006b). The presence of cyanobacterial toxins has been detected in the reservoir many times (Grabowska and Pawlik-Skowrońska 2008, Kabziński et al. 2008). Previous studies showed a very weak development of phytoplankton in the inflowing waters of the Narew

River and all small tributaries of the reservoir (Grabowska 2006a), as well as an intensive increase of phytoplankton biomass (Grabowska unpubl. data) and chlorophyll *a* concentration in the outflowing waters of the Narew River (Jekatierynczuk-Rudczyk and Górniak 2006).

The phytoplankton structure and microcystin concentrations in the Siemianówka Dam Reservoir and in the outflowing lowland Narew River were studied once a month (all stations during one day) from July to October 2008. Only in August investigations were carried more frequently and water samples were collected on four succeeding days (18–21 August). Eight sampling stations were located on the river and one station on the dam (Fig. 1). The river sampling stations were situated between 9.1 and 132.6 km downstream from the dam (Table 1). The four later river stations were situated in the Narew National Park. The samples were taken from the upper water layer (5–50 cm) from the dam (in reservoir, station No1) and at the midstream from bridges at the river stations. At each station, water temperature was measured with Hydrolab DataSonde 4 (USA). Additionally, the river flow rate was measured from bridges, using the floats.

Phytoplankton

Water samples (500 ml) for phytoplankton studies were immediately fixed using Utermöhl solution. Quantitative analyses were conducted in Fuchs-Rosenthal chambers, the counts included up to 200 individuals. The biomass of algae was determined using the volume of phytoplankton cells, measured by the author of this study.

Analysis of microcystins (MCs)

Sample preparation

Water samples (500 ml – 1500 ml) collected from the reservoir and the river were filtered onto a 47-mm glass-fiber filter disc (Whatman GF/C). Prior to extraction and analysis, the filters were stored at -20°C. Methanol (90%) extracts of the material were prepared with 15-min bath sonication (Sonorex, Bandelin, Berlin, Germany) followed by 1-min probe sonication with an HD 2070 Sonopuls ultrasonic disrupter equipped with a MS 72 probe (Bandelin, Berlin, Germany; 20 kHz, 25% duty cycle). After centrifugation at 10,000g for 15 min, the supernatant was transferred to a chromatographic vial.



Fig. 1. Map of the study sites with the location of the sampling points.

Table 1

Water parameters and phytoplankton biomass in the Siemianówka Dam Reservoir (station 1) and the Narew River (station 2-9) in July-October 2008; n.d. – not determined.

Station	Distance from dam (km)	Water temperature (°C)	Velocity (m s ⁻¹)	Total phytoplankton biomass (mg l ⁻³)	Cyanobacteria (% total biomass)	Total microcystin concentration (µg l ⁻¹)
1	0	11.0–22.8	n.d.	26.2–69.1	95.6–97.7	0.0–4.60
2	9.1	11.0–22.2	0.53–0.64	20.2–64.5	93.0–98.6	0.0–14.3
3	25.5	11.0–22.2	0.44–0.51	12.6–32.5	83.9–92.2	0.0–5.50
4	56.0	10.9–22.2	0.35–0.42	6.90–32.0	81.8–94.4	0.0–5.94
5	71.4	10.9–22.2	0.92–1.00	8.42–24.1	83.0–94.0	0.0
6	88.2	11.1–22.7	0.65–0.80	5.79–29.5	76.2–87.8	0.0–traces
7	97.6	11.0–22.0	0.25–0.37	6.38–33.0	73.7–88.2	0.19
8	107.0	10.9–22.1	0.27–0.52	7.08–29.4	60.0–88.1	0.0
9	132.6	11.0–21.7	0.21–0.30	2.60–27.7	46.5–85.9	n.d.

High-performance liquid chromatography with photodiode-array detection (HPLC-DAD)

HPLC analyses were performed in a Waters HPLC system equipped with a model 626 pump. A 996 photodiode-array detector (Waters, Milford, MA) was set at 238 nm and operated in the range 200–300 nm. Injections of 20 µl were made using a Waters 917plus autosampler. Components of the analysed extracts were separated on a Waters Symmetry RP-18 column (5 µm; 150 mm × 3.9 mm I.D.). Gradient elution with the mobile phase delivered at 1 ml min⁻¹ and consisting of 97.5% water (eluent A) and 100% acetonitrile (eluent B), both containing 0.05% trifluoroacetic acid (TFA), was used. The initial

condition was 78% of eluent A for 1 min, then the proportion of eluent B was linearly increased within 15 min to 70% and held for 1 min. After that, the proportion of B increased to 100% and was held for 2 min. Then, the initial composition of the mobile phase was reached in 5 min. MCs were tentatively identified by comparison of their retention times and UV spectra with standards purchased from Alexis Biochemicals (San Diego, CA, USA). The toxins' concentrations were quantified by calibrating them against the standard. HPLC gradient grade solvents from Baker (Deventer, The Netherlands) were used for the analyses. Water was purified to 18.2 MΩ cm (MilliQ water) using an Ultra Pure Water System from Millipore (Milford, USA).

Liquid chromatography – tandem mass spectrometry (LC-MS/MS)

The structure of microcystin variants present in cyanobacterial material was characterized by LC-MS/MS technique. The analytical system consisted of QStar Elite hybrid quadrupole-time-of-flight (Q-TOF) MS/MS with turbo ion spray (Applied Biosystems MDS Sciex, Concord, ON, Canada) and Agilent 1200 HPLC (Agilent Technologies, Waldbronn, Germany). Separation was performed on a Synergy Fusion-RP18 column (5 μm ; 50 mm \times 2.0 mm) (Phenomenex, Torrance, CA, USA). Gradient elution with a mixture of mobile phase A (5% acetonitrile containing 0.1% formic acid) and B (100% acetonitrile containing 0.1% formic acid) was used. Phase B was linearly increased from 0% to 40% in 15 min and then to 50% in 1 min. Further increase of phase B to 100% took 1 min; then it was held for 3 min and brought back to 0% B in 1 min. The column oven temperature was 35°C, the flow rate was 0.3 ml min⁻¹ and the injection volume was 5 μl . Mass spectra were acquired over the range 100 - 1100 Da with scan time of 1.0 s. The QTOF instrument was operated in the positive ion mode. Turbo ion spray (400°C) voltage was 5.5 kV, with the nebulizer gas pressure and curtain gas pressure set at 45 and 35 p.s.i (1 p.s.i. = 6894.76 Pa), respectively. Structural elucidation was achieved using collision-induced dissociation (CID), with collision energy of 50 eV, and nitrogen collision gas pressure of 6 p.s.i. Data acquisition and processing were accomplished using Analyst QS 2.0 software.

RESULTS

At sampling stations, in the reservoir and in the river, the values of water temperature were very similar (Table 1). The highest ones ($\geq 22^\circ\text{C}$) were recorded in August, the lowest in October (10.9°C - 11.1°C). Water velocity ranged from 0.21 m s⁻¹ to 1.00 m s⁻¹ (av. 0.5 m s⁻¹). The highest values of velocities were recorded at the station No. 5, the lowest - at the last three riverine stations (Table 1).

Eight major taxonomic groups of algae occurred in phytoplankton of the reservoir and its outflow. Representatives of Cyanobacteria (Cyanophyta, Cyanoprokaryota), Bacillariophyceae, Chlorophyceae and Cryptophyta were always recorded. Other four groups (Dinophyta, Euglenophyta, Chrysophyceae, Zygnematophyceae) were occasionally observed (Fig. 2AB).

The highest total biomass of phytoplankton (>60 mg l⁻¹) was recorded in July and August in the reservoir and at the nearest riverine station below the reservoir (Fig. 3). The total phytoplankton biomass was usually two-three times higher in the water collected in the reservoir (station No. 1) and at the first riverine station (No. 2) than in samples collected at the other stations (No. 3-9) (Fig. 3, Table 1). The value of the total phytoplankton biomass tends to decrease with the increasing distance from the dam. However, the dominance of cyanobacteria was always observed (Fig. 2AB, Table 1). In the reservoir, cyanobacteria reached over 95% of the phytoplankton biomass, whereas in the water at the most distant riverine stations this share decreased to 70-90%. A lower share of cyanobacteria ($\leq 60\%$) was only recorded in the water collected at the last two riverine stations (Table 1).

For the whole summer and autumn, in the water at all sampling stations, the cyanobacteria were dominated by *Oscillatoriales*. The total biomass of *Nostocales* and *Chroococcales* seldom exceeded 5% of cyanobacterial biomass. The filamentous species *Planktothrix agardhii* (Gom.) Anagn. & Kom. was the main representative of *Oscillatoriales* in all samples. The contribution of this species in cyanobacterial biomass fluctuated between 86.5%–99.9%. Thin filaments of: *Limnothrix redekei* (Van Goor) Meffert, *Pseudanabaena limnetica* (Lemn.) Kom., *Planktohyngya* spp. attained significantly lower biomass.

The green algae reached the highest biomass among other groups of the reservoir and riverine phytoplankton (Fig. 2B). *Scenedesmus*, *Monoraphidium*, *Koliella*, *Oocystis*, and *Coelastrum* were the most common genera of Chlorophyceae.

Bacillariophyceae were represented by both centric and pennate diatoms. From July to October, the biomass of centric diatoms was usually from 2 to 5 times greater than that of pennate diatoms. The first group was represented by species from genera *Stephanodiscus*, *Cyclotella*, *Aulacoseira* and *Melosira*. The second group was composed mainly of *Fragilaria ulna*, *Nitzschia* spp., *Asterionella formosa*, *Cocconeis placentula* and *Cymbella* spp.

HPLC-DAD

HPLC of water samples collected from 17th July to 13th October 2008 revealed the presence of microcystins in 12 out of 43 analyzed samples, mainly collected at stations 1-4 (Table 1). In seven samples, MC-LR was detected at concentrations ranging from

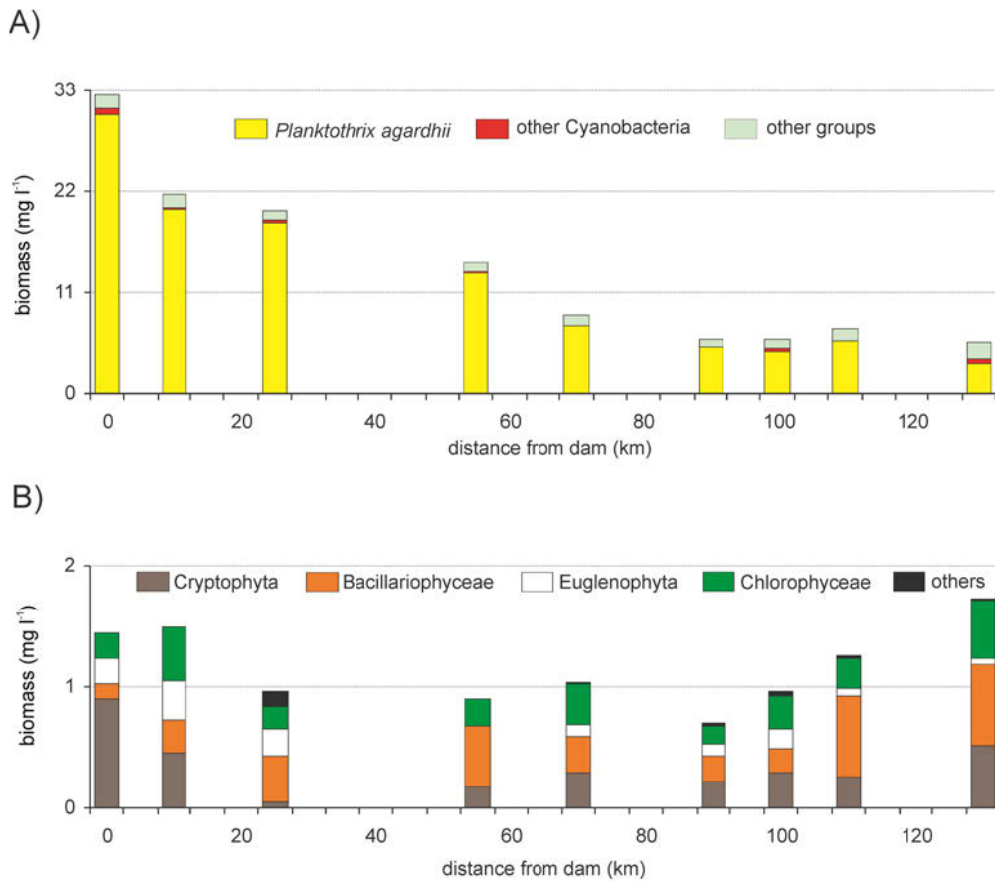


Fig. 2. Horizontal changes in the phytoplankton biomass in SDR and NR in October 2008. Total phytoplankton biomass with the marked contribution of Cyanobacteria (A); Biomass of different phytoplankton groups excluding Cyanobacteria (B). Scales of y-axis differ.

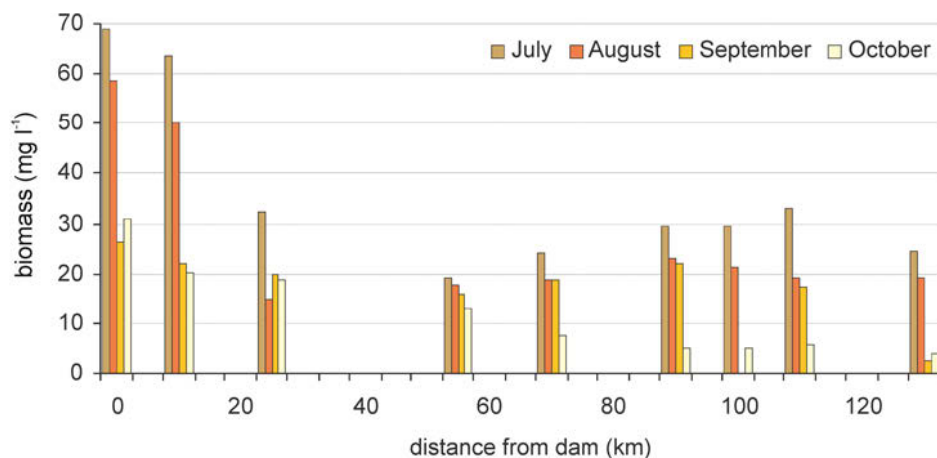


Fig. 3. Seasonal changes in total phytoplankton biomass in SDR and NR from July to October 2008.

traces to $3.80 \mu\text{g l}^{-1}$. In two samples collected in July and three samples collected in October, demethylated variants of microcystin-RR (dmMC-RR) were detected. They were present both in the

reservoir and at different stations on the river, and their concentrations were higher than those of MC-LR. In July, the measured values of dmMC-RR concentrations at station 1 and 2 were $4.6 \mu\text{g l}^{-1}$ and

5.3 $\mu\text{g l}^{-1}$, respectively; in October, they were equal to 5.25 $\mu\text{g l}^{-1}$ (25.5 km from the dam), 5.94 $\mu\text{g l}^{-1}$ (56.0 km from the dam) and 12.12 $\mu\text{g l}^{-1}$ (9.1 km from the dam). In two samples, the presence of dmMC-YR was revealed by HPLC at concentrations of 2.54 $\mu\text{g l}^{-1}$ and 4.26 $\mu\text{g l}^{-1}$.

LC-MS/MS

Analyses using the hybrid quadrupole-time-of-flight mass spectrometer confirmed the presence of demethylated variants of microcystin-RR in the extracts of cyanobacterial field samples (Fig. 4., Table 2). Based on double charge ions $[\text{M}+2\text{H}]^+$ at mass-to-charge (m/z) values 521.8, 512.8, 505.8 and 526.8, and based on the fragmentation spectra of the molecular ions, the structures of the compounds were deduced to be $[\text{Ser}^7]\text{MC-RR}$, $[\text{D-Asp}^3]\text{MC-RR}$, $[\text{D-Asp}^3, \text{Dha}^7]\text{MC-RR}$, $[\text{D-Asp}^3, \text{ADMAdda}^5]\text{MC-RR}$, respectively. ADMAdda stands for Adda⁵ residue with acetoxyl group at C-9 (C_{20} β -amino acid, (2S,3S,8S,9S)-3-amino-9-acetoxy-2,6,8-trimethyl-10-phenyldeca 4(E),6(E)-dienoic acid). Additionally, the production of demethylated variant of MC-YR ($[\text{D-Asp}^3]\text{MC-YR}$) was proved.

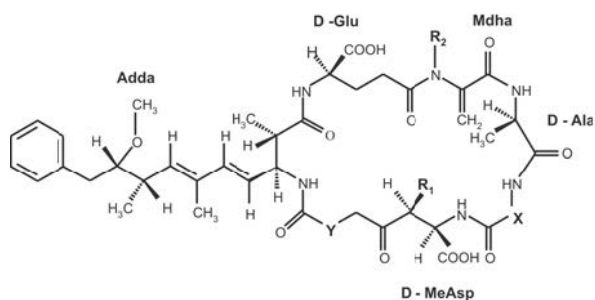


Fig. 4. Chemical structure of microcystin.

DISCUSSION

Our studies showed higher biomass of cyanobacteria in the phytoplankton of the Narew River than it was reported for other larger European rivers (Skidmore et al. 1998, Bahnwart et al. 1999, Everbecq et al. 2001, Karrasch et al. 2001, Sabater et al. 2008, Szeląg-Wasilewska et al. 2009). Species composition in the outflowing Narew River at the distance of 130 km below the dam was very similar to that in the shallow SDR. This similarity indicates a significant role of the reservoir in shaping the riverine phytoplankton. The Narew River is strongly regulated by the dam. Long retention time (av. 4–6 months) and high concentrations of nutrients stimulate the strong increase in cyanobacterial biomass in SDR (Grabowska 2005, 2006b). Due to the release of eutrophic water from the dam, the outflowing river exports a high load of phytoplankton at long distance. The results obtained in the current studies showed a decrease in the phytoplankton biomass downstream the river. The dominance of filamentous cyanobacteria, mainly from *Oscillatoriales*, was documented in samples collected at all investigated stations. The important role of the dam reservoir in shaping the phytoplankton structure was proven by Szeląg-Wasilewska et al. (2009) and Sabater et al. (2008). This was also supported by lower concentrations of chlorophyll *a* and lower biomass of phytoplankton in the twelve tributaries which join the Narew River between SDR and the Narew National Park than in the main river (data not presented).

In SDR and the Narew River, low-light adapted filamentous cyanobacteria, mainly *Planktobrix agardhii*, dominated in summer and autumn. Mass occurrence of *P. agardhii* was commonly observed in other turbid, hypertrophic or eutrophic, well-mixed lakes and dam reservoirs (Köhler and Hong 2000, Pawlik-Skowrońska et al. 2004, 2008) or lowland

Table 2

Chemical structure and toxicity of microcystin (MC) analogues identified in the cyanobacterial bloom sample collected from the Siemianówka Dam Reservoir.

Structure of microcystin analogue	X ²	Y ⁴	R ₁	R ₂	m/z [M+H] ⁺ /[M+2H] ²⁺ *	LD ₅₀ ($\mu\text{g kg}^{-1}$)
$[\text{Ser}^7]\text{MC-RR}$	Arg (R)	Arg (R)	CH ₃	CH ₃	1042/521.8*	n.a.
$[\text{D-Asp}^3]\text{MC-RR}$	Arg (R)	Arg (R)	H	CH ₃	1024/512.8	250
$[\text{D-Asp}^3]\text{MC-YR}$	Tyr (Y)	Arg (R)	H	CH ₃	1031	n.a.
$[\text{D-Asp}^3, \text{Dha}^7]\text{MC-RR}$	Arg (R)	Arg (R)	H	H	1010/505.8*	n.a.
$[\text{D-Asp}^3, \text{ADMAdda}^5]\text{MC-RR}$	Arg (R)	Arg (R)	H	CH ₃	1052/526.8*	200

*[M+2H]²⁺; n.a. – not active

polluted rivers (Köhler and Hong 2000, Karrasch et al. 2001).

In this study, *P. agardhii* was the dominant cyanobacterial species in all water samples in which microcystins were detected. Other species from *Oscillatoriales* co-occurred from July to October. Species of the genera *Anabaena*, *Microcystis* (both prolific MC producers), *Aphanizomenon*, and *Snowella* were very seldom and recorded in small numbers. Despite this similarity in the structure of the cyanobacterial community, our investigation revealed the presence of microcystins only in 12 out of 43 analyzed samples. The toxins were detected mainly in samples characterized by higher cyanobacteria biomass. It is also possible that the presence or absence of microcystins, during *P. agardhii* bloom formation in SDR and NR, results from the coexistence of MC-producing and non-MC-producing strains, which can be morphologically identical (Mbedi et al. 2005).

Planktothrix blooms occur mainly in the temperate regions of the Northern hemisphere. Due to the production of toxic metabolites, they are usually associated with the increased threat to the health of humans and animals. Microcystins are the best recognized group of active compounds derived from this genus (Sivonen and Jones 1999). *Planktothrix* tends to produce mainly demethylated microcystin variants (Fastner et al. 1999, Kurmayer et al. 2004, Welker et al. 2004). The concentrations of the toxins per dry weight is usually higher than during *Microcystis* blooms. However, studies by Yepremian et al. (2007) proved a weak relationship between microcystin concentration and the biomass of *P. agardhii*. Our results also revealed a lack of correlation between *P. agardhii* biomass and the total concentration of microcystin ($r = -0.25$, $p=0.484$).

During 2005/2006 a shift in the SDR phytoplankton composition was observed from *Chroococcales* and *Nostococales* regime to the *Oscillatoriales* dominated community (Grabowska and Pawlik-Skowrońska 2008). *Oscillatoriales*, mainly *P. agardhii*, permanently dominated over other cyanobacteria during summer and autumn in the next two years. The mass development of *P. agardhii* was concomitant with the presence of intracellular (Grabowska and Pawlik-Skowrońska 2008) and extracellular (Kabziński et al. 2008) microcystins. The highest toxin concentrations (173.8 $\mu\text{g MC-LR equiv. l}^{-1}$) were recorded in the SDR in autumn, at lower water temperatures (10.4°C) measured in the study by Grabowska and Pawlik-Skowrońska (2008). The

results of the present investigation confirmed this relation. The maximum total microcystin concentration was measured in October (14.3 $\mu\text{g l}^{-1}$) at the lowest water temperature (11.0°C). Between 1992-2003, the dominance or co-dominance of *Oscillatoriales*, were recorded rarely. Cyanobacteria blooms were most frequently comprised of the genera *Microcystis*, *Anabaena* or *Aphanizomenon*. High contribution of *Oscillatoriales* was observed in July 1999, May-August 2001 and in November 2003 (Grabowska 2005, 2006b). In 2004, *Oscillatoriales* was an important group of cyanobacteria during all seasons (Grabowska 2006b).

In flushed systems, planktonic species are potentially selected by physical factors like retention time of water, availability of light, intensity of turbulent mixing and nutrient supply. Presumably, the slowly flowing river creates similar conditions as those prevailing in the polymictic reservoir (av. velocity 0.5 m s^{-1} (Table 1). In the Narew River, high concentration of nutrients (av. 0.2 TP mg l^{-1} , av. TN 3.0 mg l^{-1}) was recorded at its long distance (Jekatierynczuk-Rudczyk and Górniak 2006). The resemblance in plankton composition in the shallow reservoir and the outflowing lowland river might be caused by similar environmental conditions, i.e. high nutrient concentration, which favors the same phytoplankton species in both types of ecosystems (Reynolds 1994). The decrease in the biomass of filamentous cyanobacteria and the shift in phytoplankton dominance to diatoms could be expected only if the concentration of nutrients in the dam is reduced. That situation was observed by Köhler and Hong (2000) in both eutrophic and polymictic Lake Müggelsee in Berlin and in the inflowing lowland Spree River.

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